Tissue and Cell 49 (2017) 106–111

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

Unilaminar follicular cells transiently express galectin-3 during ovarian folliculogenesis in pigs

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ARTICLE INFO

Article history:
Received 6 August 2016
Received in revised form 24 October 2016
Accepted 4 November 2016
Available online 17 November 2016

Keywords:
Galectin-3
Pig
Ovary
Folliculogenesis

ABSTRACT

The localization of galectin-3, a β-galactoside-binding animal lectin, was immunohistochemically studied in the ovaries of pigs to determine its expression in ovarian folliculogenesis. Various stages of ovarian follicles were identified in the ovaries of adult pigs. Galectin-3 was immunostained in the squamous follicular cells surrounding oocytes in primordial follicles and in the unilaminar granulosa cells of primary follicles, but not in oocytes of multilaminar follicles (including primary, secondary, and tertiary Graafian follicles). As in adult ovaries, galectin-3 immunoreactivity was prominent in the unilaminar follicles in neonatal ovaries. Galectin-3 was also immunolocalized in the luteal cells in the corpus luteum and granulosa cells of atretic follicles as well as in interstitial macrophages in porcine ovaries. Collectively, these results suggest that galectin-3 is transiently expressed in follicular cells in the unilaminar ovarian follicles (primordial and primary) but not in multilaminar ovarian follicles (primary to tertiary), implying that galectin-3 is embryologically involved in ovum generation.

1. Introduction

Galectin-3, a β-galactoside-binding animal lectin, has carbohydrate-recognition domains and plays a variety of roles in processes such as growth regulation, cell adhesion, cell migration, and immune responses (Barondes et al., 1994),and shows the dual immunostaining patterns in both of intracellular and extracellular components (Hughes, 1997; Liu et al., 2002). Intracellular galectin-3 plays a pivotal role in cell growth, anti-apoptosis, and mRNA splicing (Liu et al., 2002), while extracellular galectin-3 mediates inflammation and leukocyte adhesion and is involved in neutrophil recruitment and activation (Almkvist and Karlsson, 2004). Galectin-3 is also involved in the production of progesterone hormone and in the cell cycles of both luteal cells and ovary tissue of mice (Nio-Kobayashi and Iwanaga, 2010) and regressing luteal cells in bovines, processes that are influenced by prostaglandin F2 alpha (Hashiba et al., 2014). Furthermore, galectin-3 was intensively expressed in the mature mouse ovary during the luteolytic and atretic stages (Kim et al., 2012). Although galectin-3 expression has been studied in luteal cells (Hashiba et al., 2014; Kim et al., 2007; Nio-Kobayashi and Iwanaga, 2010) and the outer epithelium of female reproductive organs including the bovine uterus (Kim et al., 2008), little is known about the expression profile of galectin-3 in the course of folliculogenesis from primordial to tertiary follicles.

In porcine ovaries, follicle waves during the estrous cycle do not occur; this ensures that many follicles develop, including primordial, primary, secondary, and tertiary follicles, providing the high ovulation rate typical in pigs (Evans, 2003). With respect to ovarian enzyme expression in animals, many enzymes including nitric oxide synthase in pigs (Ding et al., 2012; Kim et al., 2005) have been differentially identified in various types of porcine ovarian follicles, suggesting that these molecules are involved in follicle development. Furthermore, galectin-3 has been consistently identified in macrophages in the ovaries of mice (Kim et al., 2007) and cows (Kim et al., 2008).

Although galectin-3 is involved in the regression of luteal cells in mice (Nio-Kobayashi and Iwanaga, 2010) and cows (Hashiba et al., 2014) as well as in atretic follicles in cows (Kim et al., 2008), its expression pattern in porcine ovaries remains unclear.

We investigated the immunoreactivity of galectin-3 in porcine ovaries at different follicular developmental stages to elucidate its role in folliculogenesis in pigs.

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http://dx.doi.org/10.1016/j.tice.2016.11.003
0040-8166/

2. Materials and methods

2.1. Animals

Neonatal (1 day old, n = 3) and adult (6 months old, n = 3) pigs were obtained from a local farm and slaughterhouse, respectively. The ovaries of the neonatal pigs were used to evaluate the primordial and unilaminar primary ovarian follicles, although these follicles are also detected in adult ovaries (however, very few if any are present in adults). All experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals at Jeju National University, Jeju, Korea.

2.2. Tissue preparation

Ovaries were dissected and fixed in 10% buffered formalin for 48 h, embedded in paraffin wax, and processed for histological examination. Paraffin sections (5 μm thick) were used for staining with hematoxylin and eosin and immunohistochemistry.

2.3. Follicle classification

Ovarian follicles were divided into the following classes as described in our previous study: (1) unilaminar follicles, including primordial follicles containing one layer of squamous cells and primary follicles containing one layer of cuboidal cells; (2) multilaminar follicles with multiple granulosa cell layers, including multilaminar primary, secondary, and tertiary follicles with an antrum; and (3) atretic antral follicles with a fragmented granulosa cell layer.

2.4. Antibodies used in this study

The rat anti-galectin-3 monoclonal antibody (1 mg/mL) was purified from the supernatant of hybridoma cells (TIB-166; ATCC, Rockville, MD, USA). Rabbit polyclonal anti-proliferating cell nuclear antigen (PCNA) (Santa Cruz Biotechnology, Dallas, Texas, USA) and ionized calcium binding protein-1 (Iba-1) (Wako Pure Chemical Industires, Ltd., Osaka, Japan) antibodies were also used.

2.5. Immunohistochemistry

Briefly, sections (5 μm thick) of paraffin wax-embedded samples were deparaffinized following routine protocols before exposing them to citrate buffer (0.01 M, pH 6.0), and then heated in a 700 W microwave oven for 3 min. All subsequent steps were performed at room temperature. The sections were treated with 0.3% hydrogen peroxide in methyl alcohol for 20 min to block endogenous peroxidase activity. After three washes in phosphate-buffered saline (PBS), the sections were blocked with 10% normal goat serum (Vector ABC Elite Kit, Burlingame, CA, USA), diluted in PBS for 1 h, and incubated with rat anti-galectin-3 (1:1000 dilution), rabbit anti-Iba-1 (1:1000 dilution), and rabbit anti-PCNA antibodies (1:200 dilution) for 1 h. After three washes in PBS, the sections were incubated with biotinylated goat anti-rat IgG (Vector), diluted 1:100, for 45 min. After three washes in PBS, the sections were incubated with the avidin–biotin peroxidase complex (Vector), prepared according to the manufacturer's instructions, for 45 min. The peroxidase reaction was developed using a peroxidase substrate kit (DAB, SK-4100; Vector) according to the manufacturer's instructions. After the completion of color development, the sections were counterstained with Harris's hematoxylin (Sigma-Aldrich) for 5 s, washed in running tap water for 20 min, dehydrated through a graded ethanol series, cleared with xylene, and mounted with Canada balsam (Sigma-Aldrich). The staining intensity was evaluated under an Olympus microscope. Three blinded observers examined three different sections from each of three animals.

3. Results

3.1. Galectin-3 immunoreaction in ovaries of neonatal pigs

Oocytes with no follicular cells gathered and formed egg nests in the cortex region of the ovary (Fig. 1A). Many unilaminar primordial and primary follicles were present with multiple rows below egg nests. Some multilaminar primary follicles were identified in the deep cortex, but secondary follicles with an antrum had not developed by 1 day after birth.

Many oocytes in egg nests were not positive for galectin-3 while some others were (Fig. 1B, arrows). In unilaminar follicular cells, an intense immunoreaction was seen in primordial follicles (Fig. 1C, arrowheads) and primary follicles with unilaminar cuboidal cells (Fig. 1C, arrows), but no oocytes exhibited a positive reaction. The granulosa layer in the multilaminar primary follicles showed a faint positive reaction (Fig. 1D, arrow). Oocytes were negative for galectin-3 in multilaminar primary follicles. Spindle-shaped cells in the stroma showed a positive reaction (Fig. 1D, arrowheads).

In the unilaminar follicular cells, galectin-3-positive cells such as unilaminar squamous cells (Fig. 2A, arrow) and cuboidal cells (Fig. 2B, arrowheads) were shown PCNA-positive immunoreaction (Fig. 2B). These results suggested that follicular cells undergo proliferation with intracellular galectin-3.

Galectin-3 immunoreaction in ovaries of adult pigs

In the adult ovary, all types of follicles including unilaminar primordial and primary follicles as well as multilaminar primary to Graafian follicles were identified in sections stained with hematoxylin and eosin.

Like neonatal ovaries, adult ovaries showed galectin-3 immunostaining in unilaminar follicular cells in primordial follicles (Fig. 3A, arrows) and in unilaminar follicular cells of primary follicles (Fig. 3A, arrowheads). However, galectin-3 was not detected in the granulosa layers of multilaminar primary follicles (Fig. 3B) or secondary (Fig. 3C) or Graafian follicles (Fig. 3D). The theca externa of secondary and Graafian follicles showed a positive reaction (Fig. 3C and D).

Galectin-3 was intensely immunostained in degenerating granulosa cells, the theca interna, and the theca externa in atretic follicles (Fig. 3E), and clearly immunostained in the luteal cells of the corpus luteum (Fig. 3F). In the stroma of the adult ovary, galectin-3 was immunostained in some cells morphologically resembling macrophages (Fig. 3B, arrows). In the serial sections, galectin-3-positive cells in Graffian follicle (Fig. 4A), atretic follicle (Fig. 4B), and luteal cell (Fig. 4C) were shown Iba-1-positive immunoreaction (Fig. 4D–F). The immunoreactivity of galectin-3 is summarized in Table 1.

4. Discussion

This is the first confirmation that galectin-3 is differentially expressed in the follicular epithelial cells of different stages of ovum development. Ova are continuously generated in the ovaries of adult pigs, which contain both unilaminar and multilaminar follicles. Ovaries are histologically classified into primordial, primary, secondary, and tertiary follicles (Sales et al., 2014), and grouped into unilaminar and multilaminar follicles based on the number of follicle-encasing cells (Sales et al., 2014). The unilaminar follicles include both primordial (ova encasing squamous follicular cells) and primary (ova encasing cuboidal cells) follicles, while multil-